実験セミナー　No. 3　　藤島利江

◎ 2-methyl-20epi 1α,25(OH)$_2$VD3誘導体の合成

<Scheme 1>

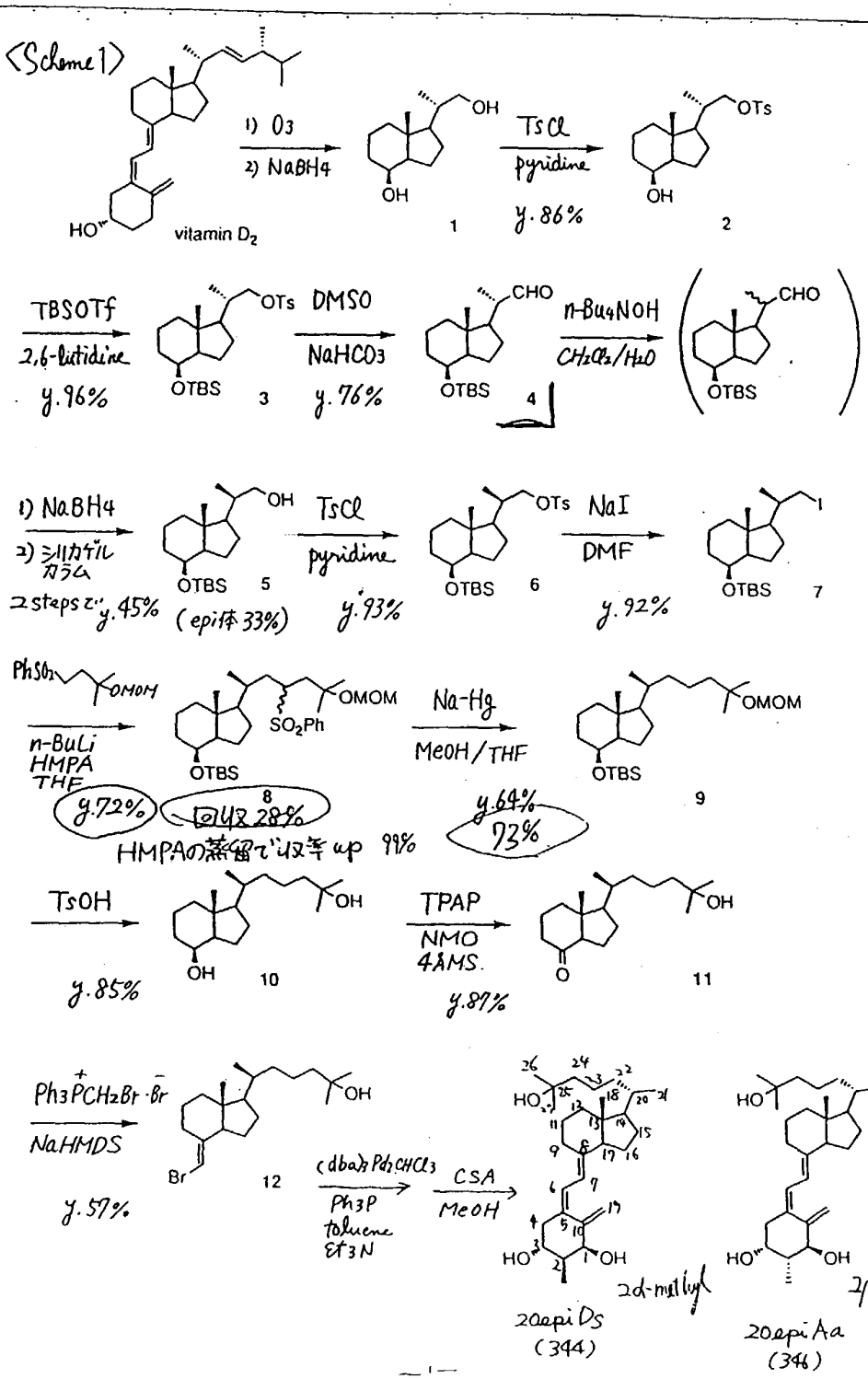


Exhibit 2
p. 1

〈Bovine Thymus VDR への結合実験〉

㊀ リン酸カリ buffer

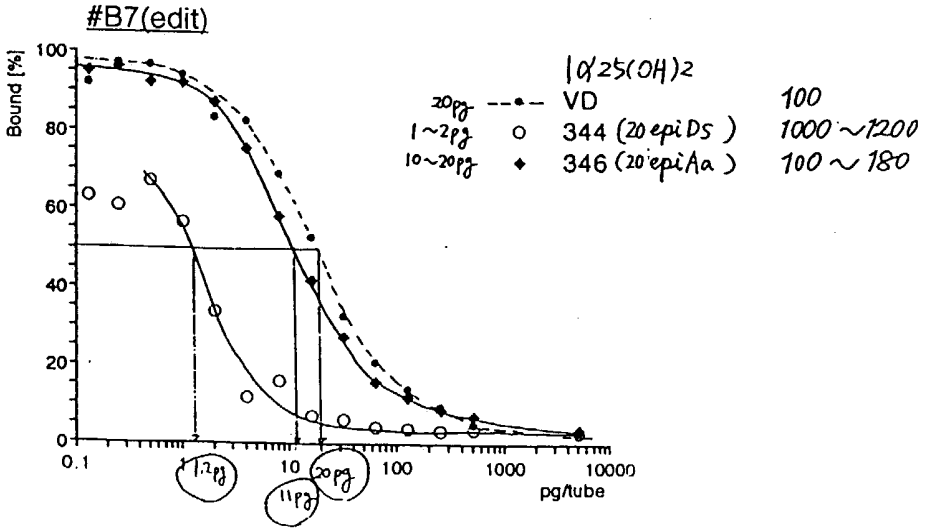
K_2HPO_4	0.05M	pH 7.4
KH_2PO_4	0.05M	
KCl	0.3M	
DTT	5mM	

㊁ 1α,25(OH)$_2$VD$_3$, #344, #346 を λmax の ε=18000 を用いて濃度調製し希釈系列を作成する.

ウシ胸腺ビタミンDレセプターはヤマサ醤油株式会社より購入し(lot.110431) 1アンプル (約25mg) を0.05Mリン酸0.5Mカリウムバッファー (pH7.4) 55 mlに溶解した。ビタミンD誘導体のエタノール溶液50 µlとレセプター溶液500 µlを室温で1時間プレインキュベートした後、1α,25-(OH)$_2$[^{3}H]VD$_3$溶液50 µlを最終濃度0.1nMとなるように加えて4℃で一晩インキュベートした。結合と非結合の1α,25-(OH)$_2$[^{3}H]VD$_3$はデキストラン-コーテド-チャコール処理して遠心分離し、上澄に液シンカクテル(ACS-II)を加えて放射活性をカウントした。

ビタミンD誘導体の活性は50%結合阻害する濃度を1α,25-(OH)$_2$VD$_3$を100としたときの比で表し評価した。

free の drug が DCC にくっついて遠沈する



cf. 20epi 1α,25(OH)$_2$VD3 の VDR への結合活性
- chicken intestine VDR 120
- bovine thymus VDR 500

Exhibit 2
p. 2

Biochemical Pharm
47(6) 987-(19

7→8 (#323)
側鎖部sulfone 980mg (3eq) in dry THF (1.5ml)を Ar雰囲気下. HMPA 1.5ml (7eq)を加え一様とした後. −78℃に冷却した。 n-BuLi (1.6M in n-hexane) 2.3ml (3eq)を滴下し −78℃で 20minかくはん後 ヨード体 7 525mg (1.20mmol) in dry THF (2+洗い込み1ml)を滴下. −78℃で1hrかくはん後 反応液に sat NH4Clを加えて EA抽出. 有機層をあわせて brineで洗い. MgSO4上 脱水. ろか. エバポレート. シリカゲルカラム (EA:n-hex=1:8)にて精製し 無色oil 8 503mg (y.72%)を得ると共に7 145mgの原料を回収(28%).

8 ^{1}H-NMR (CDCl3/TMS/400MHz) δ −0.02 (3H,s) 0.00 (3H,s) 0.66 (3H,d, J=6.4Hz) 0.85&0.88 (3H,s) 1.23&1.27 (3H,s) 2.32 (1H, dd, J=15.3Hz, 4.3Hz) 3.26 (1H,m) 3.30 (3H,s) 3.96 (1H,m) 4.57 (1H,d, J=7.3Hz) 4.67 (1H,d, J=7.3Hz) 7.55 (2H,t, J=6.3Hz) 7.63 (1H,t, J=6.3Hz) 7.88 (2H,d, J=6.3Hz)

MS = 580 (M+)

HRMS: calcd for $C_{32}H_{56}O_5SiS$ = 580.3620
found = 580.3618

8→9 (#310)
8 165mg (0.28mmol)を dry THF 3ml. dry MeOH 3mlにとかし Na_2HPO_4 3.0g. 5% Na-Hg 9.8gを加えて Ar下 rtでかくはん overnight. 反応液を etherで希釈し セライトろか. 有機層を brineで洗い. MgSO4上脱水. ろか. エバポレート. シリカゲルカラム (EA:n-hex=1:9)にて精製 9 無色oil 80mg (y.64%)を得ると共に原料 11mg (7%)を回収.

9 ^{1}H-NMR (CDCl3/TMS/400MHz) δ −0.01 (3H,s) 0.01 (3H,s) 0.81 (3H,d, J=6.7Hz) 0.89 (9H,s) 0.91 (3H,s) 1.21 (6H,s) 0.98−1.57, 1.64−1.94 (19H,m) 3.36 (3H,s) 3.99 (1H,m) 4.70 (2H,s)

MS: 440 (M+), 425 (M−Me)+

HRMS: calcd for $C_{26}H_{52}O_3Si$ = 440.3688
found = 440.3687

9 → 10 (#316)
ホゴ体 9 80mg (0.18 mmol) を MeOH 3 mlに溶かし. TsOH·H2O 174mg (0.91 mmol) を加えて rt かくはん overnight. 反応液から MeOHを エバポレートし, シリカゲルカラム (EA : nhex = 1 : 2)にて 精製. 無色 oil 43mg (y. 85%)を得る.

10 ^{1}H-NMR (CDCl3/TMS/400MHz) δ 0.84 (3H, d, J=6.7Hz) 0.93 (3H,S) 1.21 (6H,S) 4.07 (1H, m)

MS : 264 $(M-H_2O)^+$, 246 $(M-2H_2O)^+$
HRMS : calcd for $C_{18}H_{32}O$: 264.2455 $(M-H_2O)$
found : 264.2453.

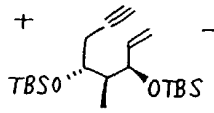
10 → 11 (#326)
アルコール 10 117mg (0.41 mmol) dry CH_2Cl_2 (10 ml). 4Å MS 30mg を Ar下 rtで 5分間かくはんする. TPAP 84mg (0.24 mmol)を加えて 1 hr 20 min 後 反応液を small pad of silica gel 上 ろかし. エバポレート. シリカゲル カラム (EA : nhex = 1 : 1)にて 精製. 100mg (y. 87%)を得る.

11 ^{1}H-NMR (CDCl3/TMS/400MHz) δ 0.64 (3H,S) 0.87 (3H, d, J=6.1Hz) 1.22 (6H,S) 2.45 (1H, dd, J=11.6Hz, 7.3Hz)

MS : 262 $(M-H_2O)^+$
HRMS : calcd for $C_{18}H_{30}O$ $(M-H_2O)$: 262.2298
found : 262.2297

11 → 12 (#334)
(bromomethyl)triphenyl phosphonium bromide 389mg (5eq) in dry THF (1.5ml)を Ar下 −60°Cに冷却し 1.0M NaHMDS 0.86ml (4.8eq) を加え −60°Cで 1hr 反応させた後. 11 50mg (0.18 mmol) in dry THF (1.5ml)に transfer する. −60°C → 0°C → rtへと 昇温し 1hr 反応させた. 反応液に n-ヘキサンを加え セライトろかし ろ液をエバポレートして シリカゲルカラム (EA : nhex = 1 : 8 → 1 : 3)にて精製. 12 36mg (y. 56%)の淡黄 oilを 得る.

12 ^{1}H-NMR (CDCl3/TMS/CDCl3) δ 0.56 (3H,S) 0.85 (3H, d, J=6.4Hz) 1.22 (6H,S) 2.88 (1H, m) 5.64 (1H, d, J=1.5Hz)
MS : 356 & 358 (M^+), 338 & 340 $(M-H_2O)^+$
HRMS : calcd for $C_{19}H_{33}O^{79}Br$: 356.1716
found : 356.1715

12 +  ⟶⟶ 20epi Ds (34a)

12 17mg (0.048mmol) を toluene 0.3mlに溶かし Et3N 0.45mlを加える. (Ar下 (dba)3Pd2·CHCl3 1.9mg (0.03eq). Ph3P 2.5mg (0.3eq.) を加え rtでかくはんしつつ A環部 13mg (0.034mmol) in toluene (150μl + 50μl)を加える. 赤黒い溶液を rtで10minかくはんすると黄色溶液となる. 120℃の oil bath上 2.5hr反応させる反応液を3か. シヨ十カラム (SiO2, EA:n-hex = 1:3)に付し 黄色oilを得る. (精製せずに次の反応へ.)

ホゴ体をMeOH 1mlにとかし CSA 11mg (0.047mmol)を加えて Ar下 rtで overnightかくはん. MeOHを留去し 水を加え EA抽出. 有キ層をあつめて brineで洗い. MgSO4上 脱水 3かエバポレート. シリカゲルカラム (EA:n-hex = 1:1)にて精製. 無色結晶 9.3mg (y.63%)を得る.

〈HPLCによる精製〉

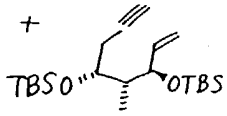
カラム: LiChrosorb RP-18 (7μm), 10×250, No.301291
溶媒: Acetonitrile : 水 = 70 : 30
recyclerをつけて 流速7.0ml/min

UV(EtOH): λmax 266nm
λmin 226nm
$\frac{A\lambda}{A\lambda_{max}} = 0.57$

^{1}H-NMR ($CDCl_3$-D_2O/TMS/400MHz) δ 0.53 (3H, s) 0.85 (3H, d J=6.7Hz) 1.08 (3H, d. J=6.8Hz) 1.21 (6H, s) 1.12—2.04 (19H, m) 2.23 (1H, dd, J=7.9Hz, 13.4Hz) 2.67 (4.0Hz, 13.4Hz) 2.83 (1H, m) 3.83 (1H, ddd, J=7.9, 4.4, 4.0Hz) 4.29 (1H, d, J=3.3Hz) 5.01 (1H, d, J=1.8Hz) 5.28 (1H, m) 6.01 (1H, d, J=11.3Hz) 6.39 (1H, d, J=11.3Hz)

MS: 430 (M^+), 412 ($(M-H_2O)^+$), 394 ($(M-2H_2O)^+$)

HRMS: calcd for $C_{28}H_{46}O_3$: 430.3447
found : 430.3443

12 +  ⟶⟶ 20epi Aa (346)

12 15mg (0.042mmol) を toluene 0.3ml に溶かし Et3N 0.45ml を加える (Ar下) (dba)3Pd2·CHCl3 1.7mg, Ph3P 2.5mg を加え rtで15分かくはんしつつ A環部 13mg (0.034mmol) in toluene (150μl+50μl) を加え 10min かくはん. 120℃の oil bath上 4hr 反応させる. 反応液をセライトろかし, ショートカラム (EA:hex = 1:3, SiO2) に付し. 黄色oilを得る.

ホゴ体を MeOH 1ml にとかし CSA 11mg (0.047mmol) を加えて Ar下 rtで overnight かくはん MeOHを溜去し. 5%NaHCO3を加え EA抽出. 有機層を brineで洗い MgSO4上脱水ろか. エバポレート. シリカゲルカラムにて (EA:n-hex=1:1) 精製後 無色結晶 4.5mg (収率31%) を得る.

<HPLCによる精製>
20epi Dsと同様の条件

UV (EtOH): λmax 263nm, λmin 228nm, $\frac{A\lambda_{min}}{A\lambda_{max}} = 0.55$

1H-NMR (CDCl3-D2O/TMS/400MHz) δ 0.55 (3H, s) 0.85 (3H, d, J=6.4Hz) 1.15 (3H, d, J=6.7Hz) 1.21 (6H, s) 1.17–2.01 (19H, m) 2.42 (1H, dd, J=13.9, 4.9Hz) 2.52 (1H, d, J=13.9Hz) 2.82 (1H, dd, J=11.9Hz, 4.0Hz) 3.99–4.04 (1H+1H, m) 5.02 (1H, t, J=1.8Hz) 5.37 (1H, t, J=1.8Hz) 6.03 (1H, d, J=11.3Hz) 6.35 (1H, d, J=11.3Hz)

MS: 430 (M+), 412 (M−H2O)+, 394 (M−2H2O)+

HRMS: calcd for C28H46O3: 430.3447
found 430.3441

English translation

Experimental Seminar No. 3

Toshie Fujishima

● Synthesis of 2-methyl-20epi 1α,25(OH)2VD3 derivatives

実験セミナー No. 3

藤島利江

◎ 2-methyl-20epi 1α,25(OH)2 VD3誘導体の合成

◎ 1α,25(OH)2 VD3のA環部の合成法

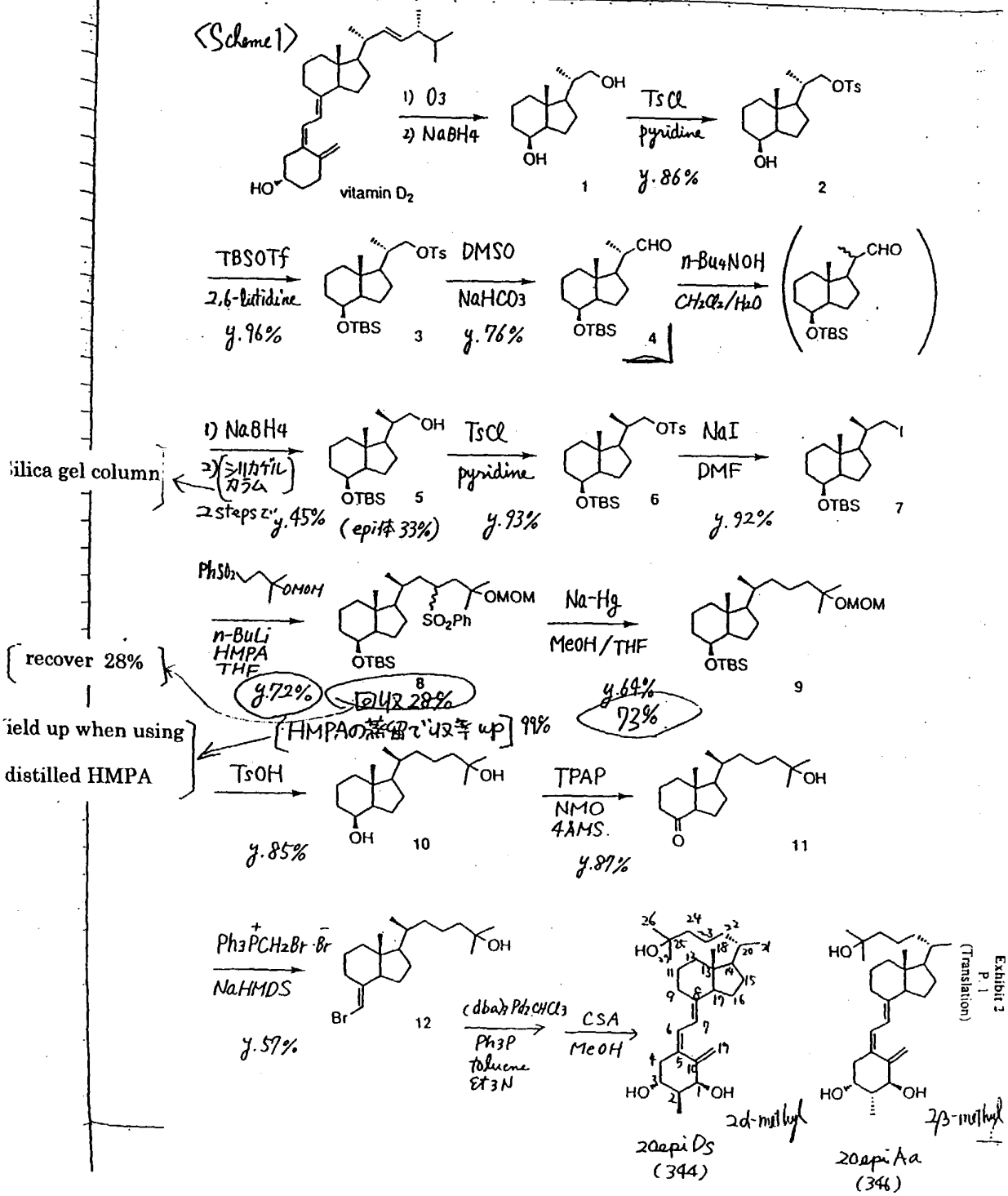


Exhibit 2
P. 1
(Translation)

Experiment of Bovine Thymus VDR binding affinity

Make diluted solution series by concentration preparation of 1α,25(OH)2VD3. #344, #346 according to λmax ε = 18000.

〈Bovine Thymus VDR への結合実験〉

㊀ リン酸カリ buffer

phosphate-potassium buffer

K_2HPO_4	0.05M	pH 7.4
KH_2PO_4	0.05M	
KCl	0.3M	
DTT	5mM	

㊁ 1α,25(OH)$_2$VD$_3$, #344, #346 と λmax の ε = 18000 を用いて濃度調製し希釈系列を作成する.

ウシ胸腺ビタミンDレセプターはヤマサ醤油株式会社より購入し(lot.110431) 1アンプル (約25mg) を0.05Mリン酸0.5Mカリウムバッファー (pH 7.4) 55 mlに溶解した。ビタミンD誘導体のエタノール溶液50 µlとレセプター溶液500 µlを室温で1時間プレインキュベートした後、1α,25-(OH)$_2$[^{3}H]VD$_3$溶液50 µlを最終濃度0.1nMとなるように加えて4℃で一晩インキュベートした。結合と非結合の1α,25-(OH)$_2$[^{3}H]VD$_3$はデキストラン-コーテド-チャコール処理して遠心分離し、上澄に液シンカクテル(ACS-II)を加えて放射活性をカウントした。

free の drug が DCC にくっついて遠沈する

ビタミンD誘導体の活性は50%結合阻害する濃度を1α,25-(OH)$_2$VD$_3$を100としたときの比で表し評価した。

The content (about 25 mg) of an ample of a Bovine Thymus Vitamin D receptor (lot. 110431), which was purchased from YAMASA SYOUYU KABUSHIKIGAISYA, was dissolved in 55 ml of a 0.05 M phosphate 0.5 M potassium buffer (pH 7.4). After pre-incubation of 50 µl of ethanol solution of Vitamin D derivative with 500 µl of receptor solution for 1 hr at room temperature, 50 µl of 1α,25-(OH)2[3H]VD3 solution was added to the pre-incubation mixture so that the final concentration became 0.1 nM and the mixture was incubated overnight at 4℃. Both of the bound and non-bound (free drug is precipitated by sticking with DCC) 1α,25-(OH)2[3H]VD3 in the mixture was centrifuged after treatment of dextran coated charcoal, liquid scintillation cocktail (ACS-II) was added to the supernatant, and the radioactivity of the resultant mixture was measured.

The binding affinity of a compound to be tested for the Vitamin D receptor was expressed by a relative intensity ratio based on 100 for 1α,25-(OH)2[3H]VD3 by determining the concentration which inhibits the binding of the hot by 50%.

Exhibit 2
P. 2
(Translation)

cf. 20epi 1α,25(OH)$_2$VD3 の VDR への結合活性
- chicken intestine VDR 120
- bovine thymus VDR 500

Binding affinity of 20-epi 1α,25-(OH)2VD3 to VDR

Biochemical Pharm
49(6) 987-(19

7→8 (#323)

側鎖部 sulfone 980mg (3eq) in dry THF (1.5ml)を Ar雰囲気下. HMPA 1.5ml (7eq)を加え一様とした後. −78℃に冷却した。 n-BuLi (1.6M in n-hexane) 2.3ml (3eq)を滴下し −78℃で 20 min かくはん後 ヨード体 7 525mg (1.20 mmol) in dry THF (2 + 洗い込み 1ml)を滴下. −78℃で 1 hr かくはん後 反応液に sat NH4Cl を加えて EA抽出. 有機層をあわせて brine で洗い. MgSO4上脱水. ろか. エバポレート. シリカゲルカラム (EA:n-hex=1:8)にて精製し無色 oil 8 503mg (y. 72%)を得ると共に 145mg の原料を回収 (28%).

Side chain sulfone 980 mg (3 eq) in dry THF (1.5 ml) was added to HMPA 1.5 ml (7 eq) under Ar atmosphere and the mixture was cooled to −78℃ after make the mixture homogeneous. n-BuLi (1.6 M in n-hexane) 2.3 ml (3 eq) was added dropwise to the mixture and stirred for 20 min at −78 ℃. Iodo form 7 525 mg (1.20 mmol) in dry THF (2 + rinse 1 ml) was dropwise added to the mixture and stirred for 1 hr at −78 ℃. Sat. NH4Cl was added to the mixture and the resultant mixture was extracted with EA. The extract was combined with organic phase and this solution was washed with brine, dried over MgSO4, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:8), 503 mg (y. 72%) of colorless oil 8 was obtained with 145 mg of the starting material 7 (28%) was recovered.

8→9 (#310)

8 165mg (0.28mmol)を dry THF 3ml. dry MeOH 3ml にとかし Na2HPO4 3.0g. 5% Na-Hg 9.8g を加えて Ar下 rt でかくはん overnight. 反応液を ether で希釈し セライトろか. 有機層を brine で洗い. MgSO4上脱水 ろか エバポレート. シリカゲルカラム (EA=n-hex=1:9)にて精製 9 無色 oil 80mg (y. 64%)を得ると共に原料 11mg (7%)を回収.

8 165 mg (0.28 mmol) was dissolved in dry THF 3 ml and dry MeOH 3 ml, Na2HPO4 3.0 g, 5% Na-Hg 9.8 g was added to the mixture and stirred overnight under Ar atmosphere at rt. The reaction mixture was diluted with ether and the resultant mixture was filtered through celite. The filtrate organic phase was washed with brine, dried over MgSO4, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:9), 80 (y. 64%) mg of colorless oil 9 was obtained with 11 mg (7%) of the starting material was recovered.

→10 (#316)
ｾｺﾞ体 9 80mg (0.18 mmol) を MeOH 3ml に溶かし. TsOH·H2O 174mg (0.91 mmol) を加えて rt かくはん overnight. 反応液から MeOH をエバポレートし シリカゲルカラム (EA:n-hex = 1:2) にて精製. 無色 oil 43mg (y. 85%) を得る.

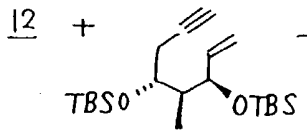
The protected form 9 80 mg (0.18 mmol) was dissolved in MeOH 3 ml, TsCl·H2O 174 mg (0.91 mmol) was added to the mixture and stirred overnight at rt. MeOH was evaporated from the reaction mixture and the residue was purified by silica gel column chromatography (EA:n-hex = 1:2), 43 mg (y. 85%) of colorless oil was obtained.

→11 (#326)
ｱﾙｺｰﾙ 10 117mg (0.41 mmol). dry CH_2Cl_2 (10 ml). 4ÅMS 30mg を Ar 下で 5分間かくはんする. TPAP 84mg (0.24 mmol) を加えて 1 hr 20 min 後反応液を small pad of silica gel 上ろかし. エバポレート. シリカゲルカラム (EA:n-hex = 1:1) にて精製. 100mg (y. 87%) を得る.

The alcohol 10 117 mg (0.41 mmol) was dissolved in CH2Cl2 (10 ml), 4ÅMS 30 mg was added to the mixture and stirred for 5 min under Ar atmosphere at rt. TPAP 84 mg (0.24 mmol) was added to the mixture and the resultant mixture was filtered through small pad of silica gel after 1 hr 20 min. The filtrate was evaporated and the residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 100 mg (y. 87%) was obtained.

→12 (#334)
(Bromomethyl)triphenyl phosphonium bromide 389 mg (5eq) in dry THF (1.5ml) を Ar 下 −60℃に冷却し 1.0M NaHMDS 0.86ml (4.8eq) を加え −60℃で 1hr 反応させた後. 11 50mg (0.18 mmol) in dry THF (1.5ml) に transfer する. −60℃ → 0℃ → rt へと昇温し 1hr 反応させた. 反応液に n-ヘキサンを加え セライトろかし ろ液をエバポレートして シリカゲルカラム (EA:n-hex = 1:8 → 1:3) にて精製. 12 36mg (y. 56%) の淡黄 oil を得る.

(Bromomethyl)triphenyl phosphonium bromide 389 mg (5 eq) in dry THF (1.5 ml) was cooled to −60℃ under Ar atmosphere and 1.0 M NaHMDS 0.86 ml (4.8 eq) was added to the mixture. The resultant mixture was reacted for 1 hr at −60℃ and the mixture was transferred to 11 50 mg (0.18 mmol) in dry THF (1.5 ml). The reaction mixture was reacted for 1 hr under the reaction temperature was warmed −60℃ → 0℃ → rt. n-Hexane was added to the reaction mixture and filtered through celite. The filtrate was evaporated and the residue was purified by silica gel column chromatography (EA:n-hex = 1:8 → 1:3), 36 mg (y. 56%) of pale yellow oil 12 was obtained.



12 17mg (0.048mmol) を toluene 0.3mlに溶かし Et3N 0.45mlを加える. (Ar下) (dba)3Pd2·CHCl3 1.9mg (0.03eq). Ph3P 2.5mg (0.3eq) を加え rtでかくはんしつつ A環部 13mg (0.034mmol) in toluene (150μl + 50μl)を加える. 赤黒い溶液を rtで10minかくはんすると黄色溶液となる. 120℃の oil bath上 2.5hr反応させる. 反応液をろか. ショートカラム (SiO2, EA=n-hex =1:3)に付し 黄色oilを得る. (精製せずに次の反応へ.)

ホゴ体を MeOH 1mlにとかし CSA 11mg (0.047mmol)を加えて Ar下 rtで overnightかくはん. MeOHを留去し 水を加え EA抽出. 有機層をあつめて brineで洗い. MgSO4上 脱水 ろかエバポレート. シリカゲルカラム (EA:n-hex=1:1)にて精製. 無色結晶 9.3mg (y.63%)を得る.

〈HPLCによる精製〉

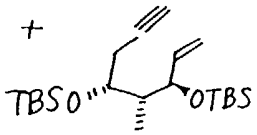
カラム: LiChrosorb RP-18 (7μm), 10×250, No.301291
溶媒: Acetonitrile : 水 = 70 : 30
recyclerをつけて 流速7.0ml/min

12 17 mg (0.048 mmol) was dissolved in toluene 0.3 ml, Et3N 0.45 ml was added to the mixture (under Ar atmosphere). (dba)3Pd2·CHCl3 1.9 mg (0.03 eq), Ph3P 2.5 mg (0.3 eq) were added to the mixture. A-ring part 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) was added to the mixture under stirring of the mixture at rt. The resultant red-black colored solution was changed to yellow solution during stirring for 10 min at rt. The resultant mixture was reacted for 2.5 hr in an oil bath at 120 ℃. The reaction mixture was filtered, the filtrate was evaporated, and the residue was treated with short column chromatography (SiO2, EA:n-hex = 1:3), yellow oil was obtained. (The next reaction was carried out without purification)

The protected form was dissolved in MeOH 1.0 ml, CSA 11 mg (0.047 mmol) was added to the mixture, and stirred overnight at rt under Ar atmosphere. MeOH was evaporated, water was added to the resultant residue and extracted with EA. The combined organic phase was washed with brine, dried over MgSO4, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 9.3 mg (y. 63%) of colorless crystal was obtained.

<Purification by HPLC>

column: LiChrosorb RP-18 (7 μm), 10 x 250, No. 301291
solvent: Acetnitrile : water = 70 : 30
flow rate 7.0 ml / min with recycler

12 +  ⟶⟶ 20epi Aa (346)

12 15 mg (0.042 mmol) を toluene 0.3 ml に溶かし Et3N 0.45 ml を加える (Ar下) (dba)3Pd2·CHCl3 1.7 mg, Ph3P 2.5 mg を加え rtで"かくはんしつつ A環部 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) を加え 10 min かくはん. 120℃の oil bath上 4hr 反応させる. 反応液をセライトろかし, ショートカラム (EA:n-hex = 1:3, SiO2) に付し. 黄色oilを得る.

ホゴ体を MeOH 1 ml にとかし CSA 11 mg (0.047 mmol) を加えて Ar下 rtで overnight かくはん MeOHを溜去し. 氷を加え EA抽出. 有機層を brineで洗い MgSO4上脱水ろか. エバポレート. シリカゲルカラムにて (EA:n-hex=1:1) 精製後 無色結晶 4.5 mg (y 31%) を得る.

<HPLCによる精製>
20epi Dsと同様の条件

12 15 mg (0.042 mmol) was dissolved in toluene 0.3 ml, Et3N 0.45 ml was added to the mixture (under Ar atmosphere). (dba)3Pd2·CHCl3 1.7 mg, Ph3P 2.5 mg were added to the mixture. A-ring part 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) was added to the mixture under stirring at rt and the mixture was stirred for 10 min. The resultant mixture was reacted for 4 hr in an oil bath at 120 ℃. The reaction mixture was filtered through celite, the filtrate was evaporated and the residue was treated with short column chromatography (SiO2, EA:n-hex = 1:3), yellow oil was obtained.

The protected form was dissolved in MeOH 1.0 ml, CSA 11 mg (0.047 mmol) was added to the mixture, and stirred overnight at rt under Ar atmosphere. MeOH was evaporated, water was added to the resultant residue and extracted with EA. The combined organic phase was washed with brine, dried over MgSO4, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 4.5 mg (y. 31%) of colorless crystal was obtained.

<Purification by HPLC>

same condition as 20 epi Ds.